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Association of Genetic Polymorphism in cyp19a Gene in Patients with Polycystic Ovarian Syndrome (PCOS) and Its Correlation with Androgen Excess in South-Indian Population

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Abstract: *This study is likely to explore the association with single nucleotide polymorphism in cyp 19a gene in patient with PCOS.*

Aim: *Investigation of genetic polymorphism in cyp19a gene in patient with PCOS.*

Setting: *Kasturba Medical College Hospital , Mangalore & Kerala Medical College & Hospital, Mangode, Cherpulassery, Palakkad Dist, Kerala.*

Methods: *103 female patients with a clinical diagnosis of PCOS attending KMC, hospital, Mangalore, medicine outpatient department & Kerala Medical College Hospital, Mangode, Cherpulassery, Palakkad Dist, Kerala. were included in the study after obtaining informed consent.*

Main Outcome & Results*: Prevalence of low physical activity, low fruit and vegetable intake, diabetes, hypertension, dyslipidimia, BMI, tobacco use, alcohol use and family history.*

Conclusion: *cyp19a gene is not associated with PCOS in South Indian population.*

Key Words: *PCOS, Genetic polymorphism*

I. Introduction

Polycystic Ovarian Syndrome (PCOS) is referred to some times as Sclerocystic Ovarian Disease, Stein-Leventhal Syndrome and Polycystic Ovarian Disease (PCOD). PCOS is a complex, heterogeneous, polygenic endocrine disorder in women of reproductive age and is considered as a multifactorial reproductive, cosmetic and metabolic problem. The etiology of PCOS is not well understood and its pathophysiological and molecular basis is still obscure. PCOS is likely to be the outcome of a number of both genetic and environmental factors. Some of the contributing factors to PCOS also include a low level of chronic inflammation in the body and fetal exposure to male hormones. However, androgen excess and insulin resistance leading to hyperinsulinemia are considered to be the basic defects in PCOS that has been described way back in 1921 by Archard & Theirs as "diabetes of bearded women"[1].

The world wide prevalence of PCOS syndrome is 6-10% and in its "classical" form may affect 5 - 7% of women[2]. PCOS is quite common in Asian population. A high prevalence of up to 35% is reported for the Indian women and the incidence and prevalence of PCOS in overweight and obese women is greater than 20%[3]. Women with PCOS are at a higher risk for a number of illnesses, including high blood pressure, diabetes, heart disease and other cardiovascular problems and cancer of the uterus, ovary and breast[4].

PCOS also presents with a variety of biochemical abnormalities[5]. The most consistent abnormality is hypersecretion of androgens. Because of the high degree of heterogeneity of PCOS, it is suggested best to consider PCOS as increased androgens clinically (acne, excessive hair on face, abdomen, or thinning of scalp hair) or in the blood (total or free testosterone, DHEAS), with oligo-ovulation (cycles greater the every 35 days, low mid-luteal progesterone, monophasic basal body temperature.

PCOS has yielded some positive results but the controversy on the mode of inheritance (eg. autosomal dominance, modified autosomal dominance, X-linked, multifactorial) still persists. Thus, there is a great need to identify the potential candidate genes that may have a modest effect individually and in groups in PCOS. Three general genetic models have been proposed namely, Single gene Mendelian model which predicts that there is single gene defect inherited in a recessive or dominant pattern and that woman who inherit this defect develop clinically evident PCOS. Multifactorial model where PCOS is considered as a multifactorial genetic disorder and women carrying this defect through inheritance or environmental factors will have increased risk of clinical

PCOS. Variable expression single gene model where there is a combination of the above two models. It follows that a single gene defect is present but its expression is modified by environmental factors. Therefore, a lady who is genetically predisposed but not exposed to environmental factors may develop only subclinical forms of PCOS and not the full disorder. This theory explains the heterogeneous nature of the disorder.

Therefore, it can be said that what started initially as a gynaecological curiosity, over the years has become a subject of multisystem endocrinopathy known as PCOS. Considering certain lacunae in the area of genetics of PCOS as pointed out earlier, we propose to study the family history of PCOS subjects, not only for PCOS but also for its related conditions. This would help us to determine the pattern of inheritance of PCOS and related clinical presentations.

Aim

To identify the frequency of the genetic polymorphisms in cyp19a gene in patients with PCOS

II. Materials and Methods

Study Setting, Design, Sampling And Period Of The Study

Out-patients and in-patients of Kasturba Medical College Hospital, Attavar, Mangalore, as well as Out-patients and in-patients of Kerala Medical College & Hospital, Mangode, Cherpulassery, Palakkad Dist Kerala diagnosed with polycystic ovarian syndrome were inducted into the study. The study was a descriptive, cross sectional type. Convenient sampling of subjects was done for the study.

Ethical Approval

Institute's Research ethical committee approval was obtained for the study. After obtaining informed consent from each participant, hundred and three (103) patients with a clinical diagnosis of PCOS (where large family trees was known) were included in the study. All patients received a long, careful and simple explanation of the purposes of the study and its pathophysiological basis.

Criteria For The Definition Of PCOS

The diagnosis of PCOS was made according to the ESHRE/ASRM criteria for the PCOS diagnosis (Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group, 2004)[6] based on the presence of two of the three following criteria: oligo- and/or anovulation (menstrual dysfunction), clinical and/or biochemical signs of hyperandrogenism and polycystic ovaries (PCO) at ultrasonogram[7].

Menstrual dysfunction was considered when the women had oligomenorrhea, defined by six or fewer cycles per year, each cycle with a length of more than 35 days, and/or when the patient had not had any menstrual bleeding for 3 consecutive months during the last year. Clinical hyperandrogenism was defined by the presence of hirsutism, represented by a hirsutism score of 8 or more. Hyperandrogenism could be clinical (hirsutism, alopecia and/or acne) or subclinical, with only an increase in serum testosterone and/or dehydroepiandrosterone sulfate[8]. Polycystic ovaries were diagnosed by pelvic sonography according to the Rotterdam conference criteria[9].

Methods

Patients And Participants

103 female patients with a clinical diagnosis of PCOS included in the study after obtaining informed consent.

Inclusion Criteria For Patients

1. Post pubertal females aged up to 35 years.
2. Irregular periods (Must have six or fewer menses /year)
3. Have clinical or laboratory evidence of hyperandrogenism (hirsutism or elevated testosterone) and PCO on ultrasound, more than 9 follicles; [Rotterdam criteria].
4. Who have signed informed consent.

Exclusion Criteria For Patients

1. Age > 35 years.
2. Diabetes Mellitus > 5 years
3. Confirmed malignancy.

103 female patients with a clinical diagnosis of PCOS by Rotterdam criteria attended KMC, hospital, Attavar; medicine outpatient department or obstetrics and gynecology department and their first and second-degree relatives; were included in the study after obtaining informed consent.

All the subjects including patients and their family members were interviewed in detail and examined for anthropometry such as BMI, Hirsutism /excess hair, Acne, Baldism, Acanthosis nigricans, Skin tags, Buffalo humps, Moon face, Double chin.

1. Biochemical assay such as; Serum fasting insulin, Cortisol, Testosterone, Dehydroxyepiandostenedione, LH, FSH,TSH were done in all cases, along with fasting lipid profile.
2. Blood pressure was measured for all, an oral 2 hr GTT was performed after 75 gm of glucose for all patients.

Genetic Anlaysis

Study of genotype and allelic frequencies were done by means of PCR- RFLP. DNA was extracted from heparinised or EDTA blood.

Ethical clearance was obtained from Manipal University institutional Ethical Committee as well as KMCH, Mangode, Cherpulassery institutional Ethical Committee after which the studies were performed.

Collection Of Blood Samples

The blood samples were collected from the patients with PCOS from coastal districts of Karnataka state and Kerala state. DNA was extracted from both test and control samples following the standard phenol-chloroform method. PCOS patients were considered as test and normal individuals of same family were used as control. Though we recruited 50 female individual devoiding of all phenotypical feature of PCOS and family history of diabetes.

III. Results

Table 1: Genotype frequencies in CYP191 genes in patients with PCOS and controls**.**

Gene	Polymorphism	Genotype frequency PCOS	Genotype frequency Controls	Chi square (X^2) P value	OR
CYP19A (RS-2414090)	$(TTTA)_n$	GG-.1 AG-.00 AA-.00	GG-.1 AG-.00 AA-.00	-	-

Table 2: Hormonal profile of polycystic ovary syndrome (PCOS) subjects.

Variable	n	Mean ± SD	Standard reference	Frequency/%
Follicle stimulating hormone (FSH)	85	5.9±1.8	3.5-12.5 µIU/ml	<3.5=6 (5) >12.5=3 (2)
Luteinizing hormone (LH)	80	10.5±9.6	2.4-12.6 µIU/ml	<2.4=3 (2) >12.6=15(14)
Testosterone (group I)	25	75.1±48.8	14-76 ng/dL	<14=2 (1) >76=8 (7)
Testosterone (group II)	45	1.3±1.4	0.08-0.48 ng/dL	<0.08=0 (0.0) >0.48=18 (17)
Dehydroepiandrosterone Sulfate (DHEAS)	80	192.1±99.7	9.4-407 mg/dL	<9.4=0 (0.0) >407=2 (1)
Thyroid stimulating hormone (TSH)	85	12.7±38.6	0.27-5.5 µIU/ml	<0.27= (0.0) >5.5=11(10)
Cortisol	85	13.3±8.2	6.4-19.4 mg/dL	<6.4=16 (15) >19.4=8 (7)
Serum insulin	72	18.9±20.5	2.6-24.9 µIU/ml	<2.6=0 (0) >24.9=5 (4)

When we screened 103 PCOS patients in cyp19a gene for -34A>G. The allele of gene CYP19A that, contain C instead of T is designated as A2 allele. The unmutated allele with T was designated as A1 allele of cyp19a gene. When we screened 103 patients with PCOS with 50 controls for the presence of polymorphic allele, No patients (0.0%) were heterozygous carrier of polymorphic A2 allele (genotype A1A2).Among 50 patients 0(0%) carried the (A2) allele in the heterozygous state (A1A2). We found no association between -34A>G polymorphism and PCOS patients.

IV. Discussion

Familial clustering of PCOS has been consistently reported, suggesting that genetic factors play a role in the development of the syndrome. Sisters, brothers, fathers, mothers, daughters and now even sons of women with PCOS have been found to have a higher risk for exhibiting either hyperandrogenic or metabolic (hyperinsulinemic) traits of the disorder and thus PCOS has become a 'family affair'[10].

In the present study we examined the prevalence of a polymorphism of gene CYP19 promoter and found no association as A1A2=0.0 and A2A2=0.0 genotype frequencies.

Echiburu et al (2008)[11] reported a frequency of 37% in PCOS subjects with A2 allele in Chilean population, when they examined 159 women with clinical and hormonal evidences of PCOS and they concluded the presence of this gene defect in PCOS seems to be associated with increase in body weight, abdominal adiposity and metabolic components.

Diamati-kandarakis et al (2009)[12] described the same gene CYP19a polymorphism in Greek population with 50 PCOS subjects and reported 58% were heterozygous carriers of the polymorphic allele and 8% carried A2 allele in homozygosity, they concluded although this base pair substitution is not a primary genetic defect in PCOS , it may aggravate in clinical picture of hyerandogenemia, particularly when homozygosity exist.

Gharani et al (1997)[13] also reported a non significant association with cyp19 gene and PCOS, when they examined 96 PCOS women in white population.

In another study Marszalek et al (2001)[14] illustrate a similar result in Poland population, while they genotyped 56 PCOS women and concluded the A>G polymorphism of cyp19 gene is not associated with steroid hormone synthesis in PCOS and it is not a primary genetic defect in this disease.

The high incidence of PCOS in first degree relatives of the affected members, in previous studies[15, 16], suggests a dominant pattern of inheritance. This is based on the assumption that at least 50% of the siblings of the PCOS probands are affected with the disorder[15]. Twin studies on PCOS have revealed an incidence of 50% has suggested a complex pattern of polygenic inheritance[17]. Other studies have reported that 50% hirsutism cases among the affected sisters of PCOS[18]. They have shown that some characteristics of PCOS inherited differed in proportion; e.g. PCO 73%, hyperandrogenemia 87% and hyperinsulinemia 66%. Another report has shown 22% of PCOS in affected sisters of the proband[18].

However when we analyzed clinical conditions associated with PCOS, we found a very high association suggesting autosomal dominant transmission. Break up data of the family history data showed nearly 20% of the fathers of pco probands having diabetes mellitus, hypertension obesity/dyslipidemia each. Also a similar percentage of mothers of the pco probands had the above metabolic syndrome characters. Among the siblings of pco probands, nearly 10% of them had diabetes mellitus, hypertension, obesity/dyslipidemia. However among the uncles, aunts and grandparents of our pco probands, the percentage of diabetes mellitus, hypertension, obesity/dyslipidemia was less than 15%. When any one of the metabolic syndrome character such as diabetes mellitus , hypertension or dyslipidemia was considered, we found prevalence of 50% and 54% among the first degree and second degree relatives of our PCOS subjects respectively.

V. Conclusion

1. Our study showed the frequency of genetic polymorphism CYP19a (0%) in patients with PCOS in South Indian.
2. We found parental metabolic syndrome related to PCOS in their offspring.

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